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EDITORIAL COMMENT

Aspirin Resistance

Transient Laboratory Finding or Important Clinical Entity?*

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For the past decade, extensive research has been devoted to study and characterize variability in the biologic response to aspirin. More than 500 publications have focused on the topic of aspirin "resistance"—low response to the antiplatelet effects of aspirin. Despite the numerous studies, substantial controversy still exists regarding the essence of this phenomenon: its definition, prevalence, potential mechanisms, and clinical implications.

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Aspirin acetylates a serine residue at position 529 on the cyclooxygenase (COX)-1 enzyme in platelets, thus blocking transformation of arachidonic acid (AA) to the potent platelet agonist thromboxane (TX) A_2 (1). Inhibition of TX synthesis and TX-dependent platelet aggregation is presumed to be the main mechanism for aspirin's antithrombotic effect (1). Response to aspirin has been evaluated by several laboratory methods (2–4). The most commonly used is light transmission aggregation in response to AA, adenosine diphosphate (ADP), or collagen (among them, AA best reflects COX-1–dependent platelet activation). A more specific method to detect COX-1 function, and therefore aspirin effect, is measurement of TXA_2 metabolites, such as serum TXB_2 and urinary 11-dehydro-TXB_2. Finally, several point-of-care assays have been developed to assess response to aspirin, such as VerifyNow Aspirin (Accumetrics Inc., San Diego, California) (2–4). The prevalence of laboratory-defined aspirin resistance (also termed low or inadequate response to aspirin) is heavily dependent on the assay used, cut-off value chosen, and population tested. A wide range of prevalence rates have therefore been reported, ranging from <1% to 60% of the subjects tested (2–4).

The clinical significance of aspirin resistance has been evaluated mainly in small studies that have used a variety of methodologies, but evidence of an association with increased risk of cardiovascular events is gradually accruing. Most of the clinical data have been attained using functional assays (e.g., platelet aggregation) or urinary 11-dehydro-TXB_2. High levels of urinary 11-dehydro-TXB_2, reflecting incomplete inhibition of TX formation by aspirin, were associated with an almost 2-fold increased risk of cardiovascular death, myocardial infarction (MI), or stroke in patients from the HOPE (Heart Outcomes Prevention Evaluation) and CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance) trials (5,6). In patients with stable coronary artery disease and patients undergoing nonurgent percutaneous coronary intervention, aspirin resistance defined by platelet aggregation (in response to AA and ADP) or the VerifyNow Aspirin assay, was associated with an increased risk of adverse events including death, MI, or stroke (7), or post-procedural myonecrosis (8,9). Finally, 2 recent meta-analyses encompassing 15 to 20 studies (albeit highly heterogeneous) and totaling almost 3,000 patients showed that aspirin resistance was associated with an odds ratio of almost 4 for development of cardiovascular events (10,11).

Despite the emerging evidence indicating an association with clinical outcomes, many fundamental questions regarding aspirin resistance are still unanswered. Of particular interest, how stable is this phenomenon temporally, and how reproducible are the results of the various assays? In this respect, the study by Santilli et al. (12) in this issue of the *Journal* adds important novel information. This comprehensive and thorough study evaluated the effect of low-dose aspirin using a variety of functional and biochemical assays in 48 healthy volunteers randomized to receive aspirin for various intervals (1 to 8 weeks). Up to 8 repeated measures of platelet function were performed in each subject, including analysis of recovery of platelet function following aspirin withdrawal. The study has 3 important findings:

1. Using any functional assay, subjects found to be resistant to aspirin at a specific time point were found to be responsive on previous or subsequent measurements. Thus, in healthy subjects, laboratory-determined aspirin resistance, assessed at a single time point, *does not* appear to be a stable phenomenon over time!
2. Aspirin induced a uniform and steady suppression of serum TXB_2 levels by ≥99% with very little interindividual or intraindividual variation (<1%). Platelet inhibition tested using AA-induced aggregation, the VerifyNow Aspirin assay, and urinary 11-dehydro-TXB_2 levels remained relatively stable, averaging 80%, 35%, and 65%, respectively, with an intrasubject variation of approximately 20% over the 8-week period. Similar TX inhibition was observed in a larger study by Faraday et al. (13) (n = 1,880 subjects without repeated measures), in which aspirin uniformly inhibited ex vivo production of TXB_2 (by ≥99%), with less pronounced inhibition of urinary 11-dehydro-TXB_2. Suppression of ADP- and

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collagen-induced aggregation was highly variable. Interestingly, there was no linear correlation between inhibition of serum TXB_2 and the other assays (including urinary 11-dehydro-TXB_2). However, AA-induced aggregation, VerifyNow Aspirin, and urinary 11-dehydro-TXB_2 levels correlated well with each other (correlation coefficients approximately 0.7), in contrast to the poor correlation found in a previous study that did not include repeated measurements (14).

3. Recovery of platelet function, assessed by the functional assays, reached approximately 70% of the baseline level at day 3 post-aspirin. This unexpectedly short period of time may have important implications for recommendations concerning aspirin withdrawal prior to surgical procedures.

The main limitation of the study by Santilli et al. (12) is that it was conducted in young (mean age 26 years), healthy individuals. Therefore, the application of these results to a population of much older patients with cardiovascular disease, comorbidities, and numerous adjunctive medications is questionable and should be further examined. Nevertheless, the interesting findings mentioned previously raise 2 broader questions:

1. Can an apparently transient unstable state, assessed using a single measurement of aspirin response, be associated with and predict potential clinical outcomes?
2. What is the reason for the nonlinear relation between serum TXB_2 levels and the functional assays, and which measure better reflects response to aspirin?

Several mechanisms for aspirin resistance have been proposed, among them poor patient compliance, inadequate aspirin dose, concomitant use of nonsteroidal anti-inflammatory drugs, rare genetic polymorphisms of COX-1, and especially relevant for the current discussion, increased platelet turnover rate and basal platelet hyper-reactivity (3,4). Both of these factors can be temporary and variable. Baseline platelet reactivity is an important determinant of post-aspirin (residual) platelet function (15,16). Increased platelet reactivity, via COX-1 and non–COX-1 pathways, can often be observed in acute conditions, such as acute coronary syndromes. Indeed, patients with acute MI display platelet hyper-reactivity and have an increased prevalence of aspirin resistance (17), which has been associated with adverse cardiac events (18). Enhanced platelet turnover, which is intertwined with platelet hyper-reactivity, causes release of young platelets still able to form TX (despite aspirin treatment) via uninhibited COX-1 and possibly through up-regulated COX-2. In both healthy subjects and patients with coronary artery disease, increased platelet turnover, as reflected by the level of reticulated platelets, has been associated with diminished response to aspirin (19,20). It is conceivable that in transient conditions associated with increased platelet turnover and reactivity, such as acute coronary syndromes or coronary artery bypass grafting, low response to aspirin may be associated with an increased risk for thrombotic complications. In such scenarios, aspirin resistance does not necessarily have to be an "all or none" constant phenotype to have clinical significance.

Regarding the second question, serum TXB_2 is the most direct and specific laboratory measure for TX generated by platelet COX-1 and therefore for COX-1 inhibition by aspirin. The nonlinear relationship between serum TXB_2 levels and other assays, also noted in previous studies (4), may reflect other sources of TX or platelet activation by other pathways. For instance, urinary 11-dehydro-TXB_2 levels may be affected by extraplatelet sources of TX, such as vascular or renal sources, or by COX-2–mediated TX generation (4). AA-induced aggregation may be affected by release of secondary agonists. Aggregation in response to ADP and collagen involves COX-1–independent pathways and possibly secondary TX-related pathways. In aspirin-treated patients, activation along these COX-1–independent pathways may contribute to residual platelet reactivity (16,21), which in turn has been associated with adverse clinical outcome (21). The question of which assay best reflects response to aspirin depends on the purpose of the test. If the aim is to predict risk of cardiovascular events in patients treated with aspirin, measurement of residual platelet reactivity (induced by AA and possibly other pathways), and urinary 11-dehydro-TXB_2 may be advantageous, as evidenced by the clinical data generated so far (5–11).

The results presented in the study by Santilli et al. (12) answer several questions but raise others. The findings regarding the transient nature of aspirin resistance should be further explored in patients with cardiovascular disease, including settings of acute coronary syndromes, percutaneous coronary intervention, and others. More importantly, a large-scale, prospective study using an acceptable common definition of aspirin resistance, preferably based on several assays, should be performed to clearly establish the long-term clinical significance of this phenomenon and accordingly the need to modify and tailor antiplatelet treatment in resistant patients.

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